PHYSICAL LIMITATIONS OF LIFE

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TABLE OF CONTENTS

Page	
1	Introduction
4	General
7	Specific Physical Boundaries
7	Water
11	Bond Cleavage Rates
11	Some Biological Effects of Deuterium
12	Essential Elements
13	Medium Composition
15	Temperature
17	Pressure
18	Concentration and Osmotic Effects
19	Energy Sources
22	Sources of Chemical and Radiant Energy
23	Thermodynamics of Biological Activity
25	Protective Mechanisms
26	Radiation
26	Magnetic and Electrical Effects
28	Gravity
28	Toxic Chemicals

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INTRODUCTION:

This project entitled "Physical Limitations of Life", Contract Number NaSr-244, commenced June 1, 1964. The stated purpose was to collect, assemble and collate data on physical limitations of life processes in order to establish the major physical boundaries within which simple life forms may exist in extra-terrestrial environments. This study is essentially restricted to microorganisms, including bacteria, molds, yeasts, algae, and certain symbiotic forms such as lichens. Although at this writing no absolute and conclusive evidence exists that life or life-like systems exist on other planets besides earth, statistics argue in favor of extra-terrestrial life. One estimate places 10^{18} planets in the universe that resemble earth and which could support earth-like life systems.

Proof for the existence of any entity based on statistics alone is always subject to criticism unless the statistical treatment is backed up by considerable experimental and observational data. The possible utility of this study, therefore, is to provide factual data on environments which might support or, at least do not exclude simple life systems.

The biosphere of the planet earth is made up of a great number of ecological zones, each of which, by virtue of their particular environmental parameters, support a specialized life system. Microorganisms are the most versatile of all living entities insofar as their ability to adapt to a great variety of environmental conditions. It is a strange paradox that microorganisms, considered the simplest forms of life, are the closest competitors to man in their ability to change their environment and themselves for their own welfare and survival. In some respects they excel man's efforts in this direction. They were the first forms to appear on earth and probably will be the last to leave the earth when the solar system runs down. The great majority of plants and animals making up the evolutionary scale between microorganisms and man have little ability to adjust to a changing environment or to change their environment for their own survival.

Like modern man, microorganisms are a powerful geologic force in their ability to affect the earth's superficial surface and atmosphere. The composition of the sedimentary materials and the atmosphere of this planet would be quite different if microorganisms had never existed. Since microorganisms are apparently essential for all forms of life, forests and other vegetation would also be non-existent and the general topography of earth would necessarily be different.

The physical manifestations of life on this planet should be apparent to an observer equipped with modern instruments on moon or mars, or an artificial satellite quite far in our solar system space. If only microorganisms and other simple forms of life existed on earth, their existence should be detectable from a distance in space because of changes produced in the chemical composition of the earth's surface.

A knowledge of the great variety of geochemical processes catalyzed by microorganisms should provide means of determining whether simple life does or has existed on near planets with powerful analytical telescopes and on distant planets with instrumented unmanned satellites. It is considered desirable, therefore, in this study to provide such information that would lead to more accurate interpretations of existing geochemical and geophysical features on other planets. It would be desirable to know, for example, whether the present Martian atmosphere reflects an ancient or existing biocatalytic activity.

Of immediate concern to our space program are possible undesirable effects resulting from "germ" contamination of other planets introduced by our space vehicles. Again, more detailed knowledge of the physical parameters in which microorganisms survive or thrive should assist our decontamination program.

Another utility of this study is to provide information for closed space habitability systems. A more complete knowledge of the chemical potential and physical limitations of microorganisms will provide more definitive guidelines for future research programs in this area, as well as discourage "blind alley" research doomed to failure because of inherent biological limitations.

The modus operandi is to establish a framework of reference upon which to build all available information on the interaction between microorganisms and their environment. This approach envisions two sets of tables for easy reference. One set will list the chemical and physical changes produced in the environment as a result of microbiological activity. The other set will list the physical environmental limitations of microbiological activity and survival.

It will be obvious to any investigator who has gone into this subject that there is now a very large fund of published information available, and that data from present research programs is coming in at an accelerated rate. The matter of evaluation becomes of critical importance when the attempt is made to distill from this mass of information the essential facts without creating erroneous ideas. For example, to report that a certain spore-forming bacterium survived autoclaving at 120° C for six hours would be misleading unless rigorous examination of the experimental conditions would rule out insulation effects. It is understood, therefore, that a supporting bibliography will be essential for a proper evaluation of the tabulated data. Further, it is recognized that this study is merely an attempt to encourage a systematic gathering of information useful for space research in the biological sciences. Ideally, to maintain a fairly complete and up-to-date set of tables as described above, a panel of experts would be needed who could monitor the research findings of the nearly 200,000 biologists existing in the world today. The most modern information gathering procedures would also be necessary, including automatic data processing.

GENERAL:

The objectives discussed in the foregoing section can be resolved into two questions: (1) Under what conditions may life exist? and (2) What visible or measurable conditions reflect life? It is obvious that answers to the first question should provide clues for the second. For this reason, the initial phase of this study was concerned primarily with delineating major boundaries of simple life systems in terms of the more critical physical limitations.

A logical starting point for this study would be that of defining a living system. As most biologists realize, this is not a simple matter. As one compares the properties of a living cell with those of inanimate systems, the distinction becomes hazy. Is the crystalline tobacco mosaic virus alive when not in the host tissue? If so, what peculiar features exist that are not found in inanimate systems?

One popular concept of life states that the secret lies in the nucleic acids, since these compounds are present in all living cells and have the ability to (1) store and pass on information, (2) effect self replication, and (3) direct energy. This definition is only partially satisfactory for we find in inorganic materials a memory system of sorts and a self replication mechanism (inorganic crystalline growth, nucleation effects of coacervates, etc.) To a degree, the specific action of inorganic catalysts is one of directing energy.

Since, at the present writing, no definition of life appears entirely satisfactory, for the purpose of this study it will be considered adequate if earth-type life is conceived as a system which contains active nucleic acids and enzymes. The term earth-type is underscored because of the possible existence of extra terrestrial life-like systems of another type. It is also recognized that the chemical composition of the earth-type life entity is one composed primarily of carbon, hydrogen, oxygen and nitrogen.

Another point of distinction to be made during this study is that between what is considered dormant life and active, metabolic life. On the one hand, information is being collected on the essential conditions for survival of a cell under a variety of environmental conditions, and on the other, conditions necessary for growth and metabolism. This distinction is readily apparent if we consider one limiting physical parameter, that of temperature. Many microorganisms can survive at the temperature of liquid nitrogen, but few can metabolize or grow when the temperature approaches zero degrees centigrade.

An operating factor which also must be considered in most of the situations describing life systems is that of time. Life processes are response-time phenomena, following thermodynamic laws. To consider the effect of temperature on living systems without the time factor becomes meaningless when we are dealing with a time range (in the space research program) from microseconds to billions of years. Life may be considered a temporary struggle against the entropy effect. The living process appears to refute certain thermodynamic laws for short periods of time, but this is a losing battle. The destiny of life, simple or complex, is to return to the disorganized or dead state. Disallowing some anthropomorphic philosophy at this point, it is sufficient to consider a life system as a transient impression on the entire cosmos. It would be interesting to speculate, then, in this study what impact life has on the cosmos, without the meddling influence of man. In other words, does non-intelligent life appear to fit in with the fundamental laws now governing the universe? Do simple life systems, as illustrated by microorganisms, appear to serve any useful purpose?

The Oparin school of thought believes that life is an inevitable chemical end point of organic synthesis in a reducing environment. Under appropriate conditions, a synthesis of a living cell from organic materials is automatic. This concept would speak for a large number of worlds in the universe populated by living organisms. Again, the purpose of life is simply one of fulfilling thermodynamic laws. If this proves to be scientifically valid, it would appear to support the existentialist school of philosophy.

Of practical import, at least as far as this project is concerned, is the argument for a large number of populated planets in the universe.

Specific Physical Boundaries:

A partial list is as follows:

Water
Essential elements
Temperature
Pressure
Concentration and Osmotic effects
Energy sources
Thermodynamics of biological activity
Protective mechanisms
Radiation, ionizing and electromagnetic
Magnetic and electrical effects
Gravity
Toxic chemicals

The first three items can be considered the most crucial for the existence of simple life forms.

Water: The importance of water to life processes is well known. Water along with carbon and nitrogen is the chemical essence of life itself. Water has many peculiar physical properties, such as high surface tension, maximum density of $4^{\circ}C$, minimum compressibility of $50^{\circ}C$, but the more indispensable components for biological systems are its solvent properties and behavior in energy reactions.

Since water is such a vital part of the living system, a great deal of research has and is being conducted to determine the precise role this compound plays in biologic processes.

The peculiar physical and chemical properties of water most probably account for many of the more well recognized manifestations of living cells such as osmotic and diffusion effects, oxidation reduction mechanisms, pH, ionization and buffering effects, biological rhymths, including vernal behavior, spore-vegetative stages, etc. Isotope studies show that water molecules are split and reassembled at a high rate in biologically active cells. The living cell has the ability to mobilize water into a lattice structure resembling liquid ice. This ordered arrangement appears to be linked to energetic mechanisms of the cell and essential for bond cleavage of the water molecule. Just how the living cell is able to orient water molecules is not clear. Some recent research suggests that water orientation results from proton concentration. Protons at high concentrations have been demonstrated to exert a strong orienting effect in ice formation.

Ice structure studies may provide important information on life mechanisms. For example, recent studies by Senftle and Thorpe at the Bureau of Standards on magnetic susceptibility of ice, show that water from cancerous and non-cancerous cells of the same tissue (rat kidney) produce different forms of ice. Normal tissue water produces hexagonal ice, while cancer tissue water produces amorphous ice. The hexagonal ice is more paramagnetic and the amorphous ice, more diamagnetic. A possible explanation for the difference in magnetic susceptibility is that the amorphous ice of cancer cells adsorbs more oxygen than the hexagonal ice, reflecting a larger percentage of unshared electrons in amorphous ice, which, in turn, indicate a breakdown of water molecule orientation.

An interesting implication of the interpretation of ice structure to space research is the possible influence of biological mechanisms. Could the structure of ice on other planets provide clues for the existence of certain life forms?

Of more practical and perhaps more immediate concern for the space program, are the preservative properties of ice on living cells. The optimum methods of freezing living cells for prolonged storage is one of the more important objectives in cryogenic research. Most cell damage resulting from freezing appears to be the result of the formation of large intracellular crystals and also the concentration of solutes that accompany ice formation. There is a great deal of variation among types of cells as to their ability to survive the freezing process. Much of the variability may simply reflect the method of freezing rather than the cells absolute ability to survive in the frozen state.

The rapid improvement in technology of preserving cells by freezing has led to some optimistic predictions for the survival time of living organisms. One author (Ettinger, R.C.W. "The Prospect of Immortality", Doubleday & Co., 1964) has predicted that man himself may be preserved indefinitely in the frozen state. This possibility, of course, is of considerable importance for extended time space flights.

Although the effect of freezing on higher animals is not within the scope of this project, the fact that such higher animals as hamsters and mice have been successfully frozen and thawed should indicate that most, if not virtually all, microorganisms can survive freezing.

The role of water in freeze drying methods is another aspect important to cell preservation. Again, technique is critical, but it does appear that many types of cells cannot survive this more drastic methodsof preservation.

The concept of panspermia, a much debated subject, is of considerable interest to many biologists who are concerned with the origin of life on earth and related phenomena. The possibility of simple life forms entering the earth from outer space rests largely on the ability of such organisms to survive for long periods of time in a frozen and dessicated state. Research into improved methods for freeze drying cells have shown that certain addatives to the cell medium enhance survival to freeze drying by providing a protective mechanism. The precise nature of the protective mechanisms is not known, although it is suspected that several factors are involved including reduction of undesirable concentrates, pH shift and large ice crystal formation. The effect of freezing and dessication on radiation damage to living cells is another important factor in long time survival of living cells in outer space or on other planets. According to some investigators the probability of lethal radiation damage mitigates against the panspermia idea. Mechanical barriers, such as would be afforded if microorganisms were inside meteors or meteor fragments, would screen out much of the radiation, but this possible mode of space travel for microorganisms is not considered the same as the panspermia concept.

The prognosis for long time survival of living cells in the frozen state or the dessicated, low temperature state must consider thermodynamic activity. There is some evidence that there may be a continous thermodynamic activity in cells at reduced temperature down to that of liquid nitrogen (-196C). Such activity virtually ceases at temperatures close to liquid helium (-269C). More research is needed to nail down this limiting factor.

The extent to which other external physical forces besides temperature affect the behavior and structure of water in the living cell is only partially understood at this writing. It is well known that infra red radiation has a marked effect on the stretching and twisting of O-H bonds. Changes in mass of the hydrogen atom by substitution of deuterium or tritium alter the bond cleavage rate of hydrogen bonds with oxygen and other atoms as indicated in the table below.

Bond Cleavage Rates at 25°C
<u>Based on Absolute-Rate Theory</u>

$$\frac{C-H}{C-D} = 7$$

$$\frac{N-H}{N-D} = 8.5$$

$$\frac{O-H}{O-D} = 10.6$$

A number of biological processes appear to be influenced by a change in mass of the hydrogen atoms in water. Some are listed in the following table:

<u>Some Biological Effects of Deuterium</u>

Toxic to mice and higher animals at concentrations above 25-30% D_2O

Low concentrations of D_2O decrease sensitivity of mice to X irradiation.

Injection of D_2O into host mice reduced growth rates of Krebs-2 as cites tumors and P-1534 lymphatic leukemia

Deuterium slowly fixed in stable positions of compounds present in tissues

Selective fractionation by some microorganisms

Most biological enzymes exhibit reduced activity

Some biological enzymes exhibit accelerated activity

Since a change in mass of one of the atoms of the water molecule results in marked alteration in biologic systems, this phenomenon poses some interesting questions and speculations. For example, by definition mass can be considered an inertial effect as well as a density effect. Any external force which can change the apparent inertia of an atom would effectively change its mass. Thru the application of forces of acceleration, magnetic or electric fields situations can be produced on bodies in which the apparent inertia can be increased or decreased. What would be the effect on biologic systems? The point of this discussion is that any environmental condition that might affect the physical properties of the liquid water molecule might also be expected to affect biologic processes. Space vehicles and other planets may be exposed to such environmental conditions not normally occurring on earth.

Essential Elements: The essential elements necessary for life may be broken down into three categories as follows:

Major	Minor	Trace			
Carbon	Potassium	Germanium	Sodium	Strontium	Mercury
Hydrogen	Magnesium	Gallium	Chlorine	Manganese	Arsenic
Oxygen	Iron	Beryllium	Iodine	Copper	Selenium
Nitrogen	Cobalt	Cadmium	Bromine	Zinc	Thallium
Phosphorus	Calcium	Cesium	Flourine	Tin	Titanium
	Sulfur	Cerium	Silicon	Aluminum	Silver
		Lanthamum	Boron	Rubidium	Molybdemum
		Thorium	Lithium	Lead	Vanadium
		Zirconium	Barium	Nickle	Radium

The major and minor elements are known to be essential for nearly all forms of life. The trace elements have been reported in various concentrations as occurring in plants and animals, but the precise role of many of these elements in biologic function is unknown. The three groupings are somewhat arbitrary and are arranged more or less in terms of relative concentration in micro-organisms and simple cell systems. The trace element list may be considered as partial.

Besides the list of essential elements, it may be useful to consider a list of essential compounds and ions. Thus, microorganisms requiring carbon dioxide for growth, may not be able to use the carbon if in the form of graphite or diamond, nor the oxygen if the only form available is in silicate. (This latter would not necessarily hold if it turns out that there exists a silicon organic system analagous to the carbon organic system on some other planet.) A comprehensive list of compounds known to be essential for all species of microorganisms would be quite large and would require a rather exhausting screening of the published literature. As an illustration, the following table lists compounds found essential for growth of one bacterial species, Clostridium sporogenes:

s-alanine	s-phenylalanine
s-valine	l-tyrosine
s-leucine	l-histidine
s-glycine	l-lysine
l-proline	d-arginine
s-aspartic acid	l-tryptophan
s-serine	thio glycolic acid
s-methionine	magnesium sulfate
l-cystine	ammonium phosphate

Besides amino acids, various salts, buffering and poising agents, many microorganisms require vitamines and miscellaneous growth factors, and of course, sources of energy such as sugars, lipids, alcohols, hydrocarbons, inorganic compounds in a reduced state, etc. Energy sources will be discussed under a separate section. At the present writing it does not appear feasible to construct a table of organic compounds essential for the great variety of microorganisms known to exist on earth. It can be assumed that virtually all naturally occurring organic molecules are utilized by microorganisms of some kind. There may be occasions, however, where the consideration of specific organic molecules as a metabolite of microorganisms is warranted. For example, the detection of methane on some planet may direct attention to the possible production or utilization of this compound by methane producing or methane oxidizing microorganisms. Of course methane along with other hydrocarbons and amino acids can be produced abiogenically from inorganic compounds by electrical discharge or ultra violet radiation. Of real interest to the matter of the origin of life and organic evolution would be the detection of complex organic molecules on other planets. Complex organic compounds in the absence of life would argue in favor of the Oparin theory of organic evolution.

One additional point in consideration of organic molecules is that such compounds in a very large variety are required by the heterotrophic microorganisms taken as a group. By definition these organisms cannot thrive in the absence of such compounds. Furthermore, the existence of heterotrophic microorganisms is virtually indicative of co-existing higher forms of plants and animals, since the latter furnish the food requirements for the former. Heterotrophs can be considered as organic scavengers. Without the more advance forms of life present, there would be nothing to scavenge. Again, a possible exception would be the situation where organic molecules might be continuously synthesized by abiogenic processes. Another possible exception would be that where autotrophs (lithotrophs and photosynthetic organisms) furnish the organic matter to the heterotrophs, either thru metabolic excretions or autolysis.

At this time, of particular interest to space biologists is the possible existence of life on Mars. From existing knowledge of the Martian environment, it seems more logical to assume that an autotrophic community, rather than a heterotrophic one, would dominate if life in any form exists there. For this reason more attention, insofar as consideration of essential elements and ions is concerned, will be given to autotrophic rather than heterotrophic life.

Temperature: Temperature limitations for microorganisms have been fairly well delineated. The maximum survival range lies between -269C and +80C. For short time periods the upper limits approach 100C. The temperature range for growth and active metabolism is much more restricted, with a minimum a few degrees above zero centigrade and a maximum around 70C. Although in recent years considerable attention has been devoted to the study of psychrophilic (cold-loving) and thermophilic (warm-loving) microorganisms, there appears to be no striking evidence that the temperature ranges cited above may be greatly exceeded by discovery of new adaptable organisms. Thermodynamic restrictions and protein denaturation have just about nailed down the upper limits, while the lower limits are just a few degrees above absolute zero. Where large fluctuations of temperature occur, on the lunar surface for example, it is the high temperatures which become limiting to microbial life in such exotic environments. There is some experimental evidence that the temperature maximum for some microorganisms may be slightly elevated by increasing the ambient pressure or thru supplying various additives to the culture medium. Aside from raising the boiling point or reducing the vapor pressure, the addatives may afford a physico-chemical protection similar to the protective colloid effect. At any rate, it does not appear likely that the upper limits of the temperature maxima now established for many thermophilic forms will be greatly exceeded in the future.

Perhaps of more interest from a space research point of view is the consideration of insulation effects afforded by natural environmental conditions. What are the diurnal temperature fluctuations a few feet below the lunar surface? In general, it may be assumed that fluctuations between extremes of hot and cold temperatures over relatively short periods of time are more conducive to life adaptation than prolonged periods of extreme temperature. Although the heat capacity of most minerals is not as high as water, considerable protection from extreme surface temperature fluctuations should be provided by this thermoregulating effect just below the surface of Earth's Moon.

The planet Venus, like the Moon, appears quite marginal for life, largely because of its high surface temperature. Again, however, insulation and thermodynamic processes, such as gas expansion, may provide localized environments where the temperatures are more conducive for earth-type life.

The equatorial region of Mars appears to be more suitable for earth-type life insofar as temperature is involved than either Moon or Venus. The surface temperature in this region may reach 25°C during daylight hours, an excellent temperature for the trowth of many microorganism. Data from land-based experiments simulating Martian diurnal temperature fluctuations should provide an educated estimate of the limiting effect of this parameter on microbial growth and metabolism.

Pr...ure: ... .mmediate interest to space biologists is the ability of microorganisms to thrive in the Martian atmosphere. The chemical composition is not well defined at this writing, although there is evidence, some much disputed, that water, carbon dioxide, compounds of nitrogen and possibly others of carbon and sulfur exist. Perhaps of greater importance for microbial life than the chemical constituents, is that of the partial pressure of the atmospheric gases. The atmospheric pressure on Mars is very low and when compared with that of earth, the concentration of the Martian gases can be considered as mere traces.

Earth-based experiments have shown that some microorganisms can survive in atmospheres approaching a pure vacuum. Active metabolism in such low pressures is another story. Moisture losses and the distillation off of essential volatiles are strongly limiting processes. There remains the not unlikely possibility of ecological niches below the Martian surface where the partial pressure of gases may be conserved and be more optimum for biological growth.

As for the upper limits of pressure, it is well established that many microorganisms on Earth are metabolically active under thousands of atmospheres of hydrostatic pressure in ocean depths. Besides high atmospheric and hydrostatic pressures, there is a substantial body of information in the published literature indicating an unusual tolerance for growth or survival by microorganisms in pure gases or gas mixtures under high pressure which are not typical of atmospheric gases in composition or relative proportion. Gases or mixtures of gases containing hydrogen, methane, carbon monoxide, hydrogen sulfide argon, neon, ammonia, oxide of nitrogen and others are tolerated by some microorganisms at high partial pressures. With the possible exceptions of some oxides of sulfur, few natural gases are universally lethal for microorganisms at moderate and high pressures.

Information is scanty on the effect of rapidly fluctuating pressures on microbial activity. It has been advanced by some investigators that pressure change rather than pressure per se is more detrimental to metabolic activity.

Concentration and Osmotic Effects: The majority of microorganisms are inhibited or killed by concentrations of salts, sugars, acids, alkalis, and other solutes in the aqueous media. Many notable exceptions exist, however. A group of organisms called halophiles are characterized by their ability to live in solutions of high salt concentrations. Several species of bacteria as well as higher organisms such as brine shrimp live in brine pools where the salts are in a saturated solution and are actually crystallizing out. A bacterium, *Thiobacillus thiooxidana*, produces sulfuric acid from free sulfur and thrives in the acid medium. As a result of the oxidation of sulfur by this organism, the hydrogen ion concentration of the aqueous medium increases to a pH of 0.5 or less. Microorganisms in general are less tolerant to extreme alkaline conditions than acid. However, there are species which ferment urea to produce ammonia and others which inhabit brine pools, salterns and bitterns, able to tolerate alkaline solutions of around pH 10. The published literature does not reveal much information on alkaline tolerance. At the present writing the upper pH limit does not appear to extend much above 10. Some land-based research would be desirable to clarify this parameter.

A number of molds tolerate high concentrations of sugar and acid. In deep culture, concentrated sugar solutives inhibit most vegetative microorganisms because of adverse osmotic effects.

Energy Sources: As mentioned previously, heterotrophic microorganisms can use as energy sources virtually all naturally occurring organic molecules. In this study, however, emphasis will be given to inorganic energy donors for microbiological processes.

The so-called autotrophs can obtain their energy from non-organic sources. A partial list of energy systems follow:

1. Chemical
2. Radiant
3. Electrical
4. Magnetic
5. Thermal
6. Mechanical

Autotrophs are known to use the first two systems in direct energy conversion processes; the third, indirectly. Virtually no information is available on the remaining three. In recent years, there has been an increasing interest in the biological effects of magnetic fields. Research has been confined largely to effects on growth, function, behavior and influence on physical properties of plants, animals, microorganisms and man. Although some research has been directed to the matter of energy transfer mechanisms, the writer is not aware of any published information on energy conversion in biological systems as such. It is understood, of course, if energy conversion in biological cells can occur thru the influence of magnetic fields, that either the field or the cell must be oscillating with respect to each other for energy to be produced. This situation is not a natural one on Earth. Its possible occurrence on other planets is considered too speculative for serious consideration at this time. As to the physiological, morphological and behavioral response of living cells to magnetic influences, this subject is recognized as an important research objective of the space program and will be considered later in this report.

Microorganisms make use of thermal energy sources for their metabolism, but not in a strict sense for energy conversion. The nearest known approximation is that of the purple sulfur bacteria which utilize infrared radiation as an energy conversion source. However, only the short wave-length in the near infrared is used for this process which places it in with the radiation category.

Mechanical energy may be considered a ridiculous possibility because living cells don't contain wheels or levers. They do possess something akin to springs, however, such as the protein helix used for motility. Some speculation has been given to the possible use of high frequency sound or ultrasonics as an energy conversion source. It is well known that sensory organs in higher animals, the lateral line in fish and the ear in other vertebrates, convert mechanical energy to electrical or electrochemical energy in the nervous system. Whether simple life forms can convert mechanical energy is quite unknown at this time. There is evidence that some single-celled forms can sense pressure changes, but this phenonmenon is not clearly recognized as an energy conversion mechanisms.

It is known that electrical energy can be used indirectly by microorganisms as a primary source for metabolic processes. Galvanic action in the soil or water which produce hydrogen thru the electrolytic dissociation of water furnish the energy for chemical conversion to a large group of microorganisms, both aerobes and anaerobes, known collectively as hydrogen-utilizing microorganisms. This group is presently under investigation for possible applications in closed-space habitability programs. Some studies have been conducted on the direct conversion of electrical to chemical energy by microorganisms. Most of this work has been done in connection with the development of the biochemical fuel cell. Bacteria have been observed growing on the surface of electrodes immersed in a simple electrolyte, such as a dilute solution of sodium chloride. The only apparent energy source was electric current. The current density was below that required for the electrolysis of water. One possible explanation of this observation is that the microorganisms, thru some unknown catalytic action, were able to reduce the activation energy at the electrode surface which enabled the electrolytic dissociation of water at lower current densities than the theoretical. Whether water dissociation, if this in fact took place, occurred internal or external to the cell was not determined. The lack of visible hydrogen bubbles suggests an internal mechanisms, although it is quite possible that the microorganisms could consume the molecular hydrogen as rapidly as it was being produced. Such an explanation has been given for the observation of a large microbial population of hydrogen - utilizers existing in marine sediments where no free hydrogen has been detected. An interesting sidelight on the subject of electrical effects of microorganisms is the probable existence of electronic analogo in microbial cells. Various organic materials exhibit semi-conductor properties. Certain cyclic biochemical processes contain enzymatic steps where the organic molecules behave as n and p type transistors. The high energy phosphate bonds behave as condensors and capacitors. The field of cybernetics which sparked the rapid development of computers and other thinking machines points the way to the eventual development of an electronic machine capable of reproducing itself; in effect, an artificial live cell. (Norbert Wiener, "God and Golem, Inc." M.I.T. Press, 1964)

Chemical and radiant energy are considered traditionally as being the principal sources for energy conversion by biological systems. Skeleton tables of these energy sources for antotrophic metabolism are presented as follows:

<u>Sources of Chemical Energy</u>

Hydrogen
Reduced iron
Free and reduced sulfur
Hydrocarbons
Ammonia
Other reduced metals?

<u>Sources of Radiant Energy</u>

Sunlight
Infrared
Ultraviolet?
Weak beta rays?

In the two tables above, all the energy sources listed with the exception of those marked questionable are known to provide energy for direct energy conversion by microorganisms and other biota. A great deal of published literature is available. One may direct attention to (1) the environmental conditions in which such energy sources may be utilized, and (2) the occurrence of these energy sources on other planets. It is anticipated that current research in these two areas will provide some answers reasonably soon. As to the questionable energy sources, these cannot be evaluated until suitable research projects are initiated.

Thermodynamics of Biological Activity: Thermodynamic treatment of biologic processes should shed light on the following: (1) utilization of energy sources as yet unknown, (2) evolution of simple life systems in non-Earth type models of exotic environments, (3) development of synthetic life systems, (4) physical parameters limiting life, e. g. temperature, pressure, concentration, etc.

The proposal for this project considered silicon as a possible substitute for carbon for a silicon analog organic system. A simple thermodynamic comparison was presented, thus

Oxide	$\Delta F\ 298^{o}$
PO_4^{---}	-241 cal.
SiO_2	-192 cal.
SO_4^{--}	-176 cal.
CO_3^{--}	-127 cal.

showing that the free energy requirements for the reduction of a silicate is not much above sulfate and carbonate which are readily reduced by microorganisms.

The fact that phosphate is used as an energy storage and energy transfer mechanisms by nearly all biologic cells suggests that silicate may be activated as well. Some investigators have postulated that silane or silicane (SiH_4) may be produced by microorganisms. Some land-based research seems warranted to verify this possibility.

The theoretical possibility of evolution of life under a variety of environmental conditions, such as those on Mars, can be approached from a thermodynamic and statistical mechanical viewpoint. According to some opinions, living organisms can be treated as, and may perhaps be, nearer to open systems than to closed systems, the classical model. Such a concept provides for greater flexibility in the thermodynamic treatment of possible life on other planets. Since the classical approach considers the closed system, whereas more recent opinions favor an open system, a compromise position would be to consider the microbial cell and its immediate environment as a closed system. Such a concept has interesting possibilities. One is the possible existence of a non-cellular life system where the immediate environmental matrix containing the molecular and ionic components form an essential part of the living mechanisms. In some respects this situation is analogous to that of a virus particle inside a host cell. In the former case, the host cell is the immediate environment. The examination of new thermodynamic concepts may assist in the eventual development of a synthetic life system as well as that of forecasting other types of life on other planets.

As this project progresses, it is anticipated that thermodynamic treatment will provide useful information on the stability of microorganisms, such as the limiting factors involved in thermal degredation of protein, etc.

Protective Mechanisms: This subject has been partially treated above under insulation effects and the discussion on water. The formation of spores by microorganisms enables these organisms to survive extreme environmental conditions. However, spore formation limits biochemical activity. Until recently, spores were considered to be chemically dormant. A recent patent describes a fermentation process in which conidia or conidio spores (commonly called spores) produce compounds in an aqueous medium without resorting to the vegetative form. The continuous catalytic conversion of one chemical substrate to other chemical products in this fermentation is evidence that all spores need not be biochemically inert. The interesting implication is that spores or other protected forms of life may be active in adverse environments such as is believed to exist on Mars.

Radiation: Ionizing and electromagnetic radiations, of particular importance in the space biology program, have been under intensive study in the past fifteen to twenty years, largely as a result of nuclear energy development. More recently, space flight programs have focused attention on the possible harmful effects of natural radiations in space on life, especially man. Microorganisms, representing fundamental biologic targets, are used as simulants for higher animals. Although it is not within the scope of this project to consider advanced life forms, experiments concerned with astronaut safety using simple life forms, promise to provide useful information on plus-minus response of microorganisms to radiation. This subject is considered vital to an understanding of biological evolution.

A crude table of the effect of short-term radiation by particulate and electromagnetic radiation, insofar as damage to single Earth-type cells can be considered, is presented below:

Alpha -	not critical
Beta -	not critical
Gamma -	damaging within limits
X-rays -	" " "
U V	damaging between 2,000 and 3,000 Å
Cosmic rays	unknown, but suspected as gene destroyers

The above list certainly requires additional qualification: Distance from radiation source, radiation barriers, condition of target, etc., should be stated for proper interpretation, and discrete numbers provided to fence in these particular parameters. Also, it must be mentioned, the situation considered here refers to autotrophs existing in the superficial surface of Earth. The table sketched out above represents a rough approximation, requiring considerable refinement for practical use, and serves only as a guide for this project.

Magnetic and Electrical Effects: A comprehensive treatment of the effect of magnetic influences on biological entities must consider qualitative field response to include homogenous and non-homogenous, alternating and static, geomagnetic and non-geomagnetic fields; quantitative aspects based on field intensity, extending from 0.5 gauss, Earth's median, to thousands of gauss; time effects; para and diamagnetic properties of the response materia; and to include the specific biological components of a living cell.

Biological response to magnetic fields are becoming increasingly more evident. Examples include: orientation effects (bird and fish navigation, plant response, microbial orientation at present is ill defined); effects on growth - the golden spiral concept; function and behavior; paramagnetic resonance involved in energy transfer; spin resonance; mutations; memory or information storage effects - analogous to ferromagnetic printing of magnetic tapes; chromosome separation; and many others.

The influence of magnetic fields on simple life systems is quite obvious. However, there appears to be a large fuzz-factor here. We know very little about the exact mechanisms which are involved. One aspect of magnetism that this writer is impressed with, insofar as simple life is concerned, is that there is no convincing evidence that biological processes can be stopped or destroyed by magnetic fields, whether they be static or alternating. For the time being, this looks like a paradox, for there are few physical parameters which, taken to extremes, can not neutralize a biological process. Gravity may be one of these.

The influence of electrical fields or currents on simple cells is also not clearly understood. However, it is possible to "electrocute" a single cell under certain conditions. Some biological species are apparently refractive to a large electric charge. Electric fish can generate pulses of current of 50 amperes and more than 500 volts. It has been postulated that single cells, such as bacteria, by virtue of their small size, conducting properties, and random motion and orientation, don't "see" electrical currents. Perhaps this is because they are not usually grounded. At any rate, it can be assumed that detrimental electrical effects to a living cell become apparent when the conditions are such that a change in chemistry, perhaps with the production of free radicals, in the environment becomes lethal to life processes.

Gravity: A big question mark is acknowledged insofar as our understanding of gravitational influences on simple cells is concerned. Theoretically, gravity has a range from zero to a very large number.

There does not appear to be substantial experimental evidence to support a concept that gravitational fields of any magnitude will limit simple life systems. Perhaps some theoretical considerations might indicate otherwise. It is acknowledged, however, that gravity affects physiological processes, but not to the extent that life becomes impossible.

Toxic Chemicals: Many chemical elements and compounds are inimical with the living cell. A table listing these materials would be quite large, and perhaps not very useful. This category of physical limitations should, for practical considerations, be confined to specific situations, as for example, the possible toxic properties of the Martian atmosphere. It is anticipated that current studies on spectral analyses by telescope and planned fly-by space-probe experiments will provide a more definitive picture of the actual composition of the Martian atmosphere. A desirable objective of this study would be to list basic principles of toxic action on simple life systems. This is a fertile, and relatively unknown field, like many others in our pursuit of the secret of life.